RESPIRATORY REGULATION IN AMPHIBIAN DEVELOPMENT [1]

JOHN R. GREGG

Zoology Department, Duke University, Durham, North Carolina

There is now considerable evidence (reviewed by Slater and Hülsmann, 1959) that the respiratory rate of a cell abundantly supplied with oxidizable substrates is a function of the rate at which it metabolizes labile phosphorus compounds in response to energy demand. Consider, for example, the oxidation of glucose:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O.$$

Normally, this process is coupled to the esterification of inorganic phosphorus:

$$C_6H_{12}O_6 + 6\,O_2 + 38\,ADP + 38\,H_3PO_4 \rightarrow 6\,CO_2 + 44\,H_2O + 38\,ATP.$$

When work is to be done, stored ATP is utilized:

$$38\,ATP + 38\,H_2O \rightarrow 38\,ADP + 38\,H_3PO_4 + Energy.$$

As a result, the levels of ADP and inorganic phosphorus are raised momentarily, and respiration is quickened until ATP is restored. Thus, under normal conditions, the respiratory rate of a cell depends upon its rate of energy expenditure.

In the presence of uncoupling agents, *e.g.*, 2,4-dinitrophenol (DNP), the link between oxidation and phosphorylation is severed. When this happens, respiration proceeds at a rate limited only by the availability of oxidizable substrates, and without concurrent formation of ATP. At the same time, ATP-stores are depleted by destructive catalysis, and the ability to perform work deteriorates rapidly.

The burgeoning respiratory rates of developing embryos surely reflect ever-increasing expenditures of energy. Therefore, a study of the respiratory responses of developing embryos to uncoupling agents should yield important information about the energetics of development. Along these lines, a recent study of sea urchin embryos by Immers and Runnström (1960) has provided interesting data, and there is reason to believe that amphibian embryos are amenable to similar analysis. In the presence of DNP, explants of the tissues of frog gastrulae respire at twice the normal rate (Ornstein and Gregg, 1952); and, under similar conditions, intact gastrulae are partially depleted of their stores of esterified phosphorus and are prevented from undergoing further morphological change (Gregg and Kahlbrock, 1957).

In the work about to be reported, an uncoupling agent (DNP) has been used to study the development of respiratory regulation in *Rana pipiens* embryos, and also in hybrid embryos obtained by fertilizing *Rana pipiens* eggs with *Rana sylvatica* sperm. First studied by Moore (1946), these hybrids are incapable of developing

[1] This work has been supported in part by a research grant, No. A-2146, from the Public Health Service. The assistance of James T. Love and B. W. Ruffner is gratefully acknowledged.

beyond the early gastrula stage, and exhibit numerous other morphogenetic or metabolic anomalies (Gregg, 1957).

Methods

Embryological

Developing embryos were obtained by stripping eggs from pituitary-injected *Rana pipiens* females into suspensions of active *Rana pipiens* or *Rana sylvatica* sperm. After two hours, they were dispersed thinly among fingerbowls and allowed to develop at temperatures ranging from 10° C. to 25° C. The medium, 10% Ringer's solution without phosphate or bicarbonate, was changed every two days, or more often. Before manometric measurements were made, watchmaker's forceps were used to free the embryos of their jelly coats.

Manometric

Respiratory rates were determined with a refrigerated Warburg respirometer equipped with 7-ml. single-side-arm center-well flasks. Carbon dioxide was absorbed on filter paper rolls placed in the center wells and saturated with 10% KOH. The flasks were shaken 75 complete cycles per minute at an amplitude of 6 cm. The temperature of the water bath was held constant at 24° C. Further details will be cited as the need arises.

Terminological

Developmental stages were determined by reference to the charts of Shumway (1940), which standardize the course of *Rana pipiens* development at 18° C. Therefore, regardless of their actual temperature histories, embryos in a given Shumway stage have been assigned the corresponding standard age at 18° C. Hybrid embryos have been assigned the same stages, and ages, as simultaneously developing *Rana pipiens* controls.

Respiratory rates are expressed in the following units: microliters of oxygen per hour per 50 embryos.

The respiratory rate exhibited by intact embryos at a given stage, and under standard conditions, is called the *respiratory norm* of embryos at that stage.

The respiratory rate exhibited by intact embryos at a given stage, and under maximal stimulation by DNP, is called the *respiratory potential* of embryos at that stage.

The quotient obtained by dividing the respiratory potential by the corresponding respiratory norm is called the *respiratory control quotient.*

Results

The results obtained in the present work are now listed without commentary. They will be discussed in the next section.

(1) The respiratory norm of *Rana pipiens* embryos is a strongly increasing function of developmental age (Table I, Table III).

(2) The relation between the developmental age and respiratory norm of *Rana pipiens* embryos is best characterized as the exponential function consisting of all pairs (t, y) satisfying the following equations:

(i)
$$y = 5\, e^{0.026t} \qquad (0 \leqslant t \leqslant 56)$$
$$y = 21\, e^{0.017\,(t-56)} \qquad (56 \leqslant t \leqslant 140)$$

TABLE I

Influence of 2,4-dinitrophenol on the respiratory rate of Rana pipiens embryos

Egg clutch	Stage	Age 18° C.	DNP-concentration, molar					
			Control	5×10^{-6}	10^{-5}	5×10^{-5}	10^{-4}	10^{-3}
A	11	34	11	19	24	33	31	—
	12½	46	18	28	35	40	35	1
	16	72	29	42	50	45	39	4
	19	118	56	93	104	85	79	25
B	6	7	6	6	13	24	23	3
	10½	30	12	16	22	32	28	2
	15	67	24	33	36	40	35	9
	10½	90	38	54	71	53	51	6
	19	118	56	85	102	71	64	26
	20	140	84	112	118	104	78	19

Main compartment of each flask: 20–50 embryos in 1 ml. 10% Ringer's. Side-arm of each flask: 0.5 ml. DNP in 10% Ringer's adjusted to give final concentration shown. Side-arm contents delivered to main compartment immediately after first reading. Readings were made for four or five hours (usually five) at half-hour intervals. Respiratory rates were constant after first hour. Entries designate average rates for last three (four) hours of four (five) hour runs.

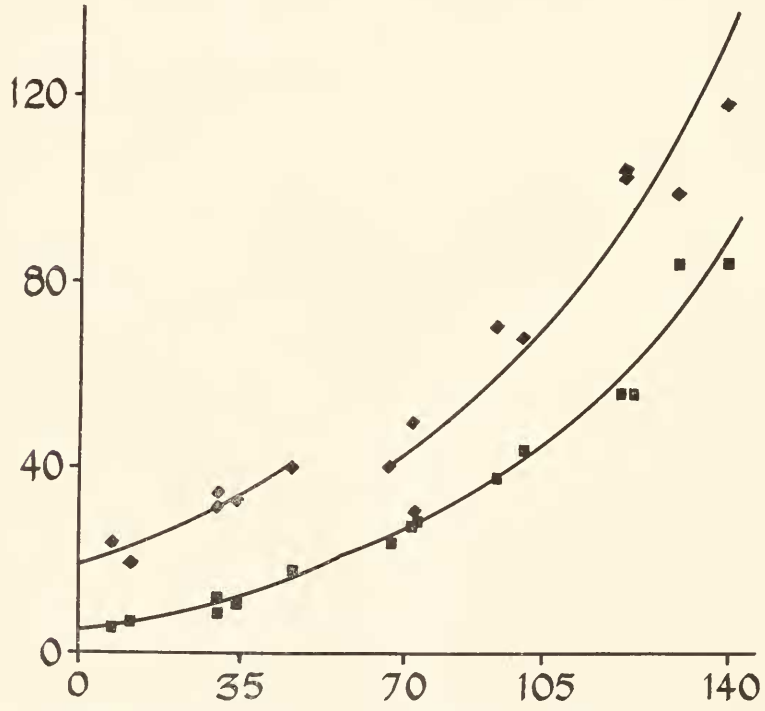

FIGURE 1. Respiratory norm (lower curve) and potential (upper curve) of *Rana pipiens* embryos. Abscissa, developmental age. Ordinate, respiratory rate.

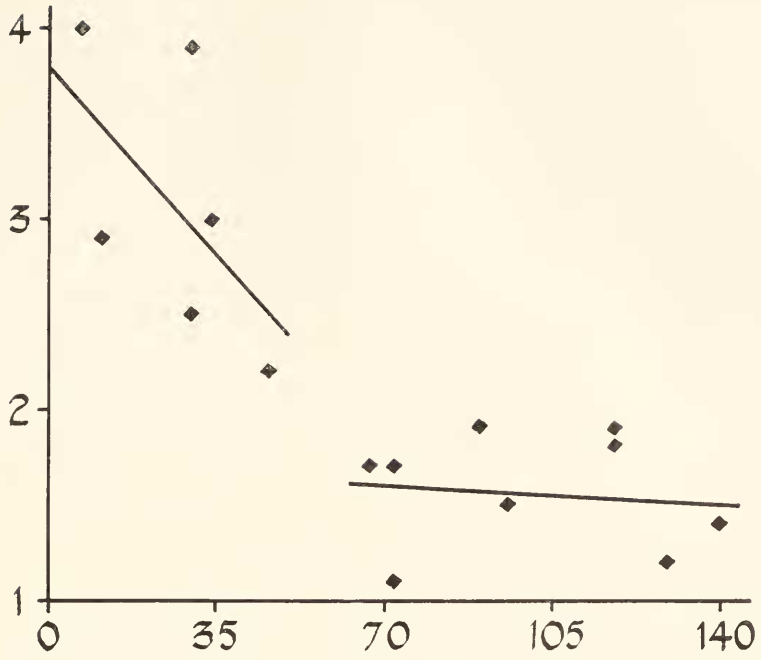

FIGURE 2. Respiratory control quotient, *Rana pipiens*. Abscissa, developmental age. Ordinate, respiratory control quotient.

TABLE 11

Influence of 2,4-dinitrophenol on the respiratory rate of Rana pipiens ♀ × Rana sylvatica ♂ embryos

Egg clutch	Stage	Age 18° C.	DNP-concentration, molar					
			Control	5×10^{-6}	10^{-5}	5×10^{-5}	10^{-4}	5×10^{-4}
C	$7\frac{1}{2}$	11	5	13	20	22	18	20
	$10\frac{1}{2}$	30	3	10	18	31	30	17
	$13\frac{1}{2}$	56	3	13	21	28	26	15
	$17\frac{1}{2}$	90	12	18	22	29	—	20
	19	118	11	19	21	35	32	32
D	7^{+}	9	8	13	20	26	28	15
	$11\frac{1}{2}$	38	9	18	25	30	28	12
	15	67	13	17	22	35	37	19
	18	96	10	18	26	38	38	28

Fifty embryos per flask. DNP-treated embryos were equilibrated in DNP-solutions in 10% Ringer's for two hours preceding measurements. Readings were taken for one hour at 5-minute intervals.

Hybrid embryos do not develop beyond Stage 10: entries in the stage and age columns designate average developmental stages and corresponding ages of *Rana pipiens* control embryos.

where t is the developmental age and y is the respiratory norm (Fig. 1, lower curve).

(3) The respiratory activity of *Rana pipiens* embryos at any stage is stimulated by DNP in concentrations ranging from 5×10^{-6} M to 1×10^{-4} M (Table I, Table III).

(4) The respiratory potential of *Rana pipiens* pre-neurulae is exhibited under treatment with DNP at concentrations near 5×10^{-5} M; that of neurulae and older embryos is exhibited under treatment with DNP at concentrations near 10^{-5} M (Table I, Table III).

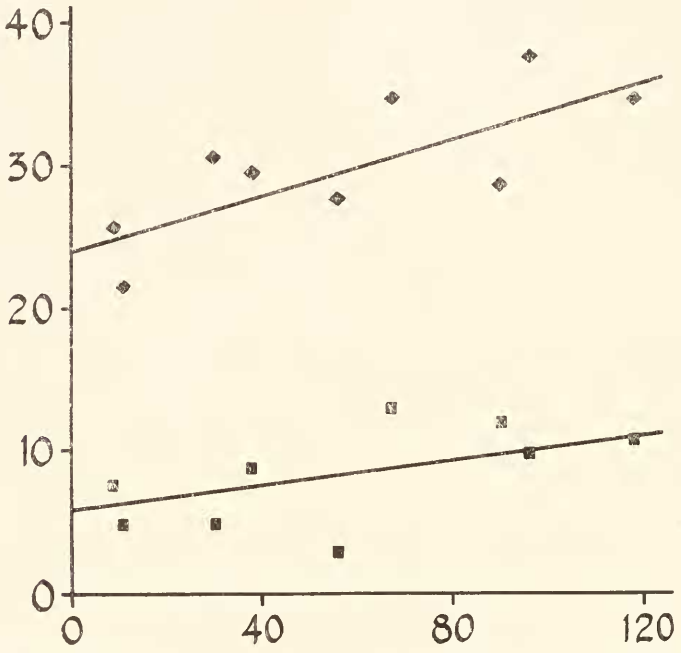

FIGURE 3. Respiratory norm (lower curve) and potential (upper curve) of *Rana pipiens* ♀ × *Rana sylvatica* ♂ embryos. Abscissa, developmental age. Ordinate, respiratory rate. (24° C.)

(5) The relation between the developmental age and respiratory potential of *Rana pipiens* embryos is best characterized as the exponential function consisting of all pairs (t, y) satisfying the following equations:

(ii)
$$y = 19\, e^{0.017t} \qquad (0 \leqslant t \leqslant 46)$$
$$y = 41\, e^{0.016\,(t-67)} \qquad (67 \leqslant t \leqslant 140)$$

where t is the developmental age and y is the respiratory potential (Fig. 1, upper curve). For reasons explained later, the respiratory potentials corresponding to some values of t $(46 < t < 67)$ are left undefined.

(6) The respiratory control quotient of *Rana pipiens* embryos decreases rapidly from 3.8 at fertilization to 2.5 at 46 hours, and slowly from 1.6 at 67 hours to 1.5 at 140 hours (Fig. 2). For reasons explained later, the respiratory control quotients of embryos between the ages of 46 hours and 67 hours are left undefined.

(7) The respiratory norm of hybrid embryos is a weakly increasing function of developmental age (Table II).

(8) The relation between the developmental age and respiratory norm of hybrid embryos is best characterized as the linear function consisting of all pairs (t, y) satisfying the following equation:

(iii) $$y = 6 + 0.045t \qquad (0 \leqslant t \leqslant 118)$$

where t is the developmental age and y is the respiratory norm (Fig. 3, lower curve).

(9) The respiratory activity of hybrid embryos at any stage is stimulated by DNP in concentrations ranging from 5×10^{-6} M to 5×10^{-4} M (Table II).

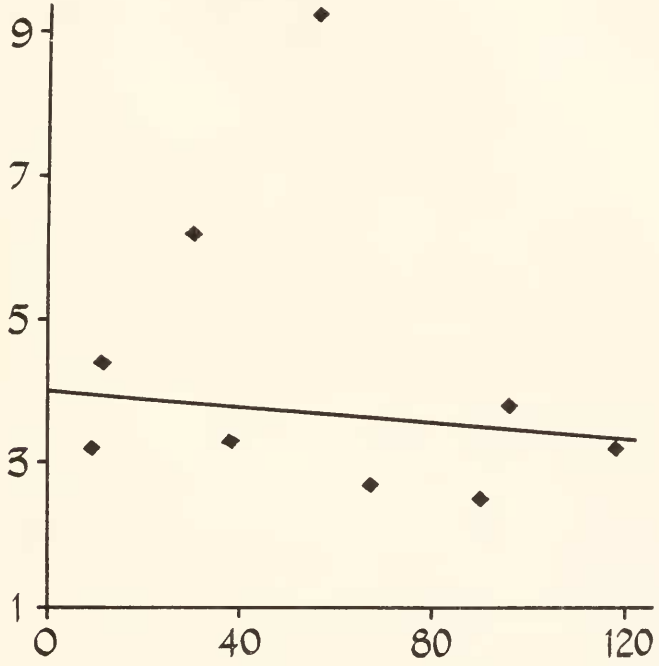

FIGURE 4. Respiratory control quotient, *Rana pipiens* ♀ × *Rana sylvatica* ♂. Abscissa, developmental age. Ordinate, respiratory control quotient.

(10) The respiratory potential of hybrid embryos is exhibited under treatment with DNP at concentrations near 5×10^{-5} M (Table II).

(11) The relation between the developmental age and respiratory potential of hybrid embryos is best characterized as the linear function consisting of all pairs (t, y) satisfying the following equation:

(iv) $$y = 24 + 0.10t \qquad (0 \leqslant t \leqslant 118)$$

where t is the developmental age and y is the respiratory potential (Fig. 3, upper curve).

(12) The respiratory control quotient of hybrid embryos decreases from 4 at fertilization to 3.3 at 118 hours (Fig. 4).

(13) Homogenization in buffer-saline solution has little effect upon the respiratory activity of young *Rana pipiens* embryos, but it powerfully stimulates the respiratory activity of older ones (Table III).

(14) The respiratory rates exhibited by homogenized *Rana pipiens* embryos are not affected by DNP in concentrations ranging from 10^{-5} *M* to 5×10^{-5} *M* (Table III).

TABLE III

Influence of 2,4-dinitrophenol on the respiratory rate of intact embryos and cell-free homogenates (Rana pipiens)

Egg clutch	Stage	Age 18° C.	DNP-concentration, molar		
			Control	10^{-5}	5×10^{-5}
E Intact	7½	11	7	—	20
	10½	30	9	—	35
	16	72	28	31	—
	18	96	44	68	—
	19½	129	84	99	—
E Homogenized	7½	11	8	6	9
	10½	30	8	10	7
	16	72	132	130	129
	18	96	132	128	124
	19½	129	146	136	137

Intact embryos. Fifty embryos per flask. DNP-treated embryos were equilibrated in DNP solutions (in 10% Ringer's) for one to two hours preceding measurements. Readings were taken for one hour at 5-minute intervals.

Homogenates. Embryos suspended in ice-cold 10^{-2} *M* phosphate buffer made up in 0.065% NaCl (50 embryos per ml.) were homogenized with a Lourdes homogenizer. Aliquots (1 ml.) were transferred to respirometer flasks. Immediately after first reading, DNP solutions (0.5 ml. in buffer-saline, "pH 7.48") were tipped into main compartments to give final DNP-concentrations desired. Readings were taken for one hour at 5-minute intervals.

Entries in the stage and age columns designate average developmental stages and corresponding ages.

DISCUSSION

The results listed in the previous section will now be discussed, in sequence.

(1) Embryologists have known for a long time that developing amphibian embryos are characterized by waxing respiratory norms. Our first result, then, is not new, and our chief concern will be to find an explanation for it.

One explanation is provided by the assumption that developmentally increasing respiratory rate is the direct result of the synthesis of respiratory machinery. But recent experiments (Spiegelman and Steinbach, 1945; Gregg and Ray, 1957) have established that homogenates of newly fertilized eggs can be made to respire endogenous substrates at rates exceeding those of intact embryos at any stage of development. From the outset, therefore, there is more than enough respiratory apparatus to support any rate of respiration normally exhibited by a developing embryo, and the suggested explanation cannot be correct.

Another explanation, sponsored by Spiegelman and Steinbach, and also by Gregg and Ray, assumes that increasing respiratory norms are the direct result of processes progressively facilitating effective contact between respiratory enzymes and their substrates, *i.e.*, that the respiratory rate at any stage is limited simply by the rate at which some respiratory enzyme is able to combine with its substrate. But, under this assumption, we are left without good reason to expect an elevation of respiratory rate in the presence of uncoupling agents, and it is best abandoned.

Still another explanation (the one we shall adopt here) is provided by the theory outlined in the introduction. According to this theory, the respiratory norm is a function of the rate of energy expenditure; and waxing respiratory norms thus are to be ascribed to waxing rates of energy expenditure. In this connection it is known that developing *Rana pipiens* embryos are characterized by increasing rates of carbohydrate utilization (Gregg, 1948), and by increasing rates of turnover of labile phosphorus (Kutsky, 1950).

(2) Our mathematical analysis of the relation between developmental age and respiratory norm (*Rana pipiens*) has been made following the precedent established by Atlas (1938) and by Moog (1944), and the results of it are in considerable agreement with theirs. The following version of their equations is obtained by changing units to correspond with those used in the present investigation and by confining values of t to the interval (0–140):

(v) Atlas	$y = 3\,e^{0.038t}$	$(0 \leqslant t \leqslant 62)$
	$y = 16\,e^{0.019\,(t-62)}$	$(62 \leqslant t \leqslant 140)$
(vi) Moog	$y = 5\,e^{0.077t}$	$(0 \leqslant t \leqslant 50)$
	$y = 21\,e^{0.031\,(t-50)}$	$(50 \leqslant t \leqslant 140)$

Considering the nature of the supporting data, (i), (v) and (vi) are in good agreement. For *Rana pipiens* embryos, therefore, it appears to be established that respiratory norm is an exponential function of developmental age, and that respiratory acceleration decreases at some point in the age interval (50–62). For possible explanations of the acceleratory change, readers are referred to the papers of Atlas and Moog.

(3) We have already mentioned that *Rana pipiens* embryos are well-provisioned with respiratory substrates; therefore, on the basis of the theory outlined in the introduction, the stimulatory effect of DNP is to be expected.

(4) What is unexpected is the finding that the concentration of DNP eliciting the respiratory potential of *Rana pipiens* embryos is five times as great for the age interval (0–67) as for the age interval (72–140). There is no parallel for it in the development of sea urchin embryos, whose respiration is maximally stimulated at any stage by 5×10^{-5} *M* DNP (Immers and Rumnström, 1960); and, pending further investigation, it remains unexplained.

(5) There is no precedent to guide mathematical analysis of the data relating developmental age to respiratory potential (*Rana pipiens*); and subsequent investigation may necessitate revision of equations (iv), which have been obtained by taking the data at face value. For what it is worth, Figure 1 (upper curve) shows that the development of the respiratory potential of *Rana pipiens* embryos proceeds in three phases: two of exponentially increasing potential, separated by one whose characteristics are not known. The second phase may be one of constant potential,

as the data suggest; or, if the first and third phases are of greater duration than shown, it may be one of abruptly decreasing potential; or, although there is no reason for so believing, later work may show that the first and third phases actually intersect in the age interval (46–67), thus abolishing the second phase entirely. We shall leave the question open, after noting that Immers and Runnström (1960) observed a transient decline of respiratory potential in sea urchin embryos entering the mesenchyme blastula stage. But their result, also, was reported in a mood of skepticism.

In any case, it is clear that the respiratory potential of *Rana pipiens* embryos increases with age, remaining well above the respiratory norm, and thus maintaining a considerable margin of safety for energy expenditure. We have accounted for the developmental increase of respiratory norm by supposing that it is a function of increasing rate of energy expenditure. To account for increasing respiratory potential, it is necessary to assume the occurrence of intracellular structural changes progressively enhancing contact between respiratory enzymes and substrates. Data bearing upon this assumption are neither crucial nor consistent. Weber and Boell (1955) have found that the specific activity of mitochondrial cytochrome oxidase is an increasing function of developmental age (*Xenopus laevis*), thus indicating some process of mitochondrial differentiation; on the other hand, Spiegelman and Steinbach (1945) were unable to observe any developmental increase of the cytochrome oxidase activity of homogenates (*Rana pipiens*). Nevertheless, our assumption is supported by the electron microscopical study of Eakin and Lehmann (1957), who discovered profound developmental alterations of structural complexity and localization of the intracellular components, including mitochondria, of the ectoderm of neurulating amphibian embryos (*Xenopus laevis, Triton alpestris*). Therefore, until better assumptions are available, we shall adhere to our present one.

(6) The respiratory control quotient is a convenient measure of the degree to which the rate of energy expenditure holds the respiratory norm below the respiratory potential. Figure 1 shows that, during the first 46 hours of development, energy expenditure in *Rana pipiens* embryos is such as to permit a rapid approach of the respiratory norm to the respiratory potential; from 67 hours on, the relation is nearly stabilized, and respiratory norm is practically a constant fraction of respiratory potential. For reasons stated in the discussion of result (5), respiratory control quotients corresponding to the age interval (46–67) are left undefined. It is worth noting that a similar relation between respiratory norm and respiratory potential characterizes the development of sea urchin embryos (Immers and Runnström, 1960).

(7) We come now to the respiration of hybrid embryos. In a general way the data agree with those of Barth (1946) in showing that the respiratory norms of such embryos become increasingly subnormal as time goes on, and the same may be said of the rates at which they utilize carbohydrate reserves (Gregg, 1948). It appears, therefore, that they expend energy at increasingly subnormal rates; and this, on the theory of respiratory control we are adopting, is the reason for their progressively subnormal respiratory norms. On this basis, we should expect to find increasingly subnormal rates of turnover in their pools of labile phosphorus, but data are not yet available. There may be nothing wrong with their respiratory machinery, for their

homogenates respire at rates quantitatively similar to those of homogenates of *Rana pipiens* control embryos (Gregg and Ray, 1957).

(8) Our mathematical analysis of the relation between the developmental age and respiratory norm of hybrid embryos is based upon the assumption of linearity. The more precise data of Barth (1946) suggest that this assumption is not quite correct, but it is a useful approximation to the exact state of affairs. It should be noted that the intercept 6 of equation (iii) is in good agreement with the intercept 5 of equation (i).

(9) The view that the respiratory machinery of hybrid embryos may be entirely normal is supported by the finding that their respiratory rates are stimulated from 300 to 400% by 5×10^{-5} *M* DNP; for no better response is obtainable from *Rana pipiens* control embryos.

(10) This result need not be elaborated, except by pointing out that the concentration of DNP eliciting the respiratory potential of hybrid embryos at any stage is the same as that eliciting the respiratory potential of *Rana pipiens* pre-neurulae: there is no developmental shift in sensitivity to DNP like that exhibited by neurulating *Rana pipiens* embryos.

(11) On the assumption of linearity, the rate at which the respiratory potential of hybrid embryos increases is given by equation (iv). The increase of respiratory potential, though slower than normal, still is unnecessary; for the respiratory norms of hybrid embryos never overtake their initial respiratory potential (Fig. 2). Nevertheless, an increase of respiratory potential occurs; and to explain it we shall assume, in accordance with the discussion of result (5), that intracellular changes facilitating respiratory enzyme-substrate union take place in hybrid embryos as well as in normal ones, though at a much slower rate. Electron microscopical and biochemical studies of the intracellular particulates of hybrid embryos are much needed.

(12) This result does not require further commentary: reference to the discussion of result (6) will make its interpretation perfectly evident.

(13) This finding confirms the work of Gregg and Ray (1957): unless they are treated with a detergent (*e.g.,* deoxycholic acid), homogenates of very young *Rana pipiens* embryos do not respire at rates much different from the respiratory norm; but the respiratory rates of homogenates of progressively older embryos rapidly exceed the respiratory norm. We shall return to this topic in the discussion of the last result.

(14) The failure of DNP to elevate the respiratory activity of homogenates of *Rana pipiens* embryos at any stage of development is extremely puzzling, and no adequate explanation for it is now at hand.

Homogenates of adult tissues frequently do not exhibit a respiratory response to the presence of uncoupling agents. In such cases, respiratory rate appears to be limited, not by the rate of turnover of labile phosphorus, but by the low availability of readily oxidizable substrates; generally, this limitation is overcome by adding pyruvate, succinate or other respiratory metabolites (Krebs, 1959). This account of the matter is not applicable to homogenates of amphibian embryos (see the discussion of result (1)).

An arbitrary explanation, for which there is little independent support, may be constructed along the following lines. First, let us suppose that homogenization, by activating ATP-ase, results in some maximum elevation of the levels of ADP and

inorganic phosphorus. Second, let us suppose that homogenization is accompanied by the envelopment of respiratory particulates in lipo-protein or other envelopes. Third, let us suppose that the degree of envelopment decreases with developmental age. The first assumption guarantees a high rate of respiratory activity, other conditions permitting; and explains why DNP is without effect, the levels of ADP and inorganic phosphorus already being maximum. The second assumption explains why the respiratory activity of homogenates of young embryos is low, for we may expect that respiration under these conditions will be limited by the rate at which respiratory substrates are able to penetrate lipo-protein barriers. It also explains why the respiratory rate of homogenates of young embryos is elevated by detergents, for these may be expected to disperse lipo-protein deposits around respiratory particulates; or, even, to fragment those particulates (Siekevitz and Watson, 1956). The third assumption explains why the respiratory activity of homogenates is an increasing function of developmental age.

Summary

1. The respiratory rate of *Rana pipiens* control embryos is an increasing function of developmental stage, with an acceleratory change at the onset of the formation of the neural folds.

2. At any stage of development, the respiratory rate of *Rana pipiens* embryos is elevated by the presence of 2,4-dinitrophenol (DNP). The degree of stimulation obtainable ranges from about 400% of the control rate at the beginning of development to about 150% of the control rate at the gill-circulation stage.

3. The respiratory rate of *Rana pipiens* ♀ × *Rana sylvatica* ♂ embryos is an increasing function of time, but the rate of increase is very much lower than that of the respiratory rate of *Rana pipiens* controls.

4. At any stage, the respiratory rate of hybrid embryos is elevated by DNP. The degree of stimulation obtainable ranges from about 400% at the beginning of development to about 300% at 118 hours after fertilization (18° C.).

5. The respiratory activity of homogenates of *Rana pipiens* embryos at any stage is not altered by the addition of DNP.

6. The relevance of these findings to the question of embryonic respiratory control is discussed. It is concluded that, within the capacity to respire, respiration is governed by energy expenditure, and that the capacity to respire increases with age as the result of intracellular changes facilitating contact between respiratory enzymes and substrates.

LITERATURE CITED

Atlas, M., 1938. The rate of oxygen consumption of frogs during embryonic development. *Physiol. Zoöl.*, **11**: 278–291.

Barth, L. G., 1946. Studies on the metabolism of development. *J. Exp. Zool.*, **103**: 463–486.

Eakin, R. M., and F. E. Lehmann, 1957. An electronmicroscopic study of developing amphibian ectoderm. *Arch. f. Entw.*, **150**: 177–198.

Gregg, John R., 1948. Carbohydrate metabolism of normal and of hybrid embryos. *J. Exp. Zool.*, **109**: 119–134.

Gregg, John R., 1957. Morphogenesis and metabolism of gastrula-arrested embryos in the hybrid *Rana pipiens* ♀ × *Rana sylvatica* ♂. *In:* The Beginnings of Embryonic Development, edited by Albert Tyler, R. C. von Borstel and Charles B. Metz, Publication No. 48 of the American Association for the Advancement of Science. Washington, D. C.

GREGG, JOHN R., AND MARGIT KAHLBROCK, 1957. The effects of some developmental inhibitors on the phosphorus balance of amphibian gastrulae. *Biol. Bull.*, **113**: 376–381.

GREGG, JOHN R., AND FRANCES L. RAY, 1957. Respiration of homogenized embryos: *Rana pipiens* and *Rana pipiens* ♀ × *Rana sylvatica* ♂. *Biol. Bull.*, **113**: 382–387.

IMMERS, J., AND J. RUNNSTRÖM, 1960. Release of respiratory control by 2,4 dinitrophenol in different stages of sea-urchin development. *Dev. Biology*, **2**: 90–104.

KREBS, HANS, 1959. Chairman's introductory address. Rate-limiting factors in cell-respiration. *Ciba Foundation Symposium on the Regulation of Cell Metabolism, pp. 1–10.*

KUTSKY, PHYLLIS B., 1950. Phosphate metabolism in the early development of *Rana pipiens*. *J. Exp. Zool.*, **115**: 424–460.

MOOG, FLORENCE, 1944. The chloretone sensitivity of frog's eggs in relation to respiration and development. *J. Cell. Comp. Physiol.*, **23**: 131–155.

MOORE, J. A., 1946. Studies in the development of frog hybrids. I. Embryonic development in the cross *Rana pipiens* ♀ × *Rana sylvatica* ♂. *J. Exp. Zool.*, **101**: 173–220.

ORNSTEIN, NORMA, AND JOHN R. GREGG, 1952. Respiratory metabolism of amphibian gastrula explants. *Biol. Bull.*, **103**: 407–420.

SHUMWAY, W., 1940. Stages in the normal development of *Rana pipiens* I. External form. *Anat. Rec.*, **78**: 139–147.

SIEKEVITZ, P., AND M. WATSON, 1956. Cytochemical studies of mitochondria. I. The separation and identification of a membrane fraction from isolated mitochondria. *J. Biophys. Biochem. Res.*, **2**: 639–652.

SLATER, E. C., AND W. C. HÜLSMANN, 1959. Control of rate of intracellular respiration. *Ciba Foundation Symposium on the Regulation of Cell Metabolism, pp. 58–83.*

SPIEGELMAN, S., AND H. B. STEINBACH, 1945. Substrate-enzyme orientation during embryonic development. *Biol. Bull.*, **88**: 254–268.

WEBER, R., AND E. J. BOELL, 1955. Über die Cytochromoxydaseaktivität der Mitochondrien von frühen Entwicklungsstadien des Krallenfrosches (*Xenopus laevis* Daud.). *Rev. Suisse de Zool.*, **62**: 260–268.